A NOTE ON THE SYSTEMATIC POSITION OF *MYCOBACTERIUM COELIACUM*.

By H. L. Jensen.
Macleay Bacteriologist to the Society.

(One Text-figure.)

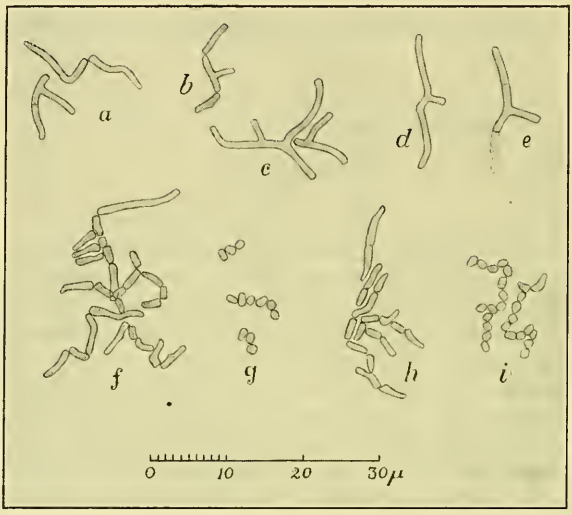
[Read 27th May, 1931.]

Mycobacterium coeliacum was described by Gray and Thornton (1928) in a study of soil bacteria capable of decomposing aromatic compounds. Twenty-five species, belonging to seven genera, were described; among these were six species of *Mycobacterium*. The authors point out that their classification is merely temporary, since our present knowledge is insufficient to enable us to classify the bacteria satisfactorily. In the most recent edition of Bergey's Manual of Determinative Bacteriology (1930) the mycobacteria described by Gray and Thornton have been transferred to other genera—five to the genus *Actinomyces*, and one, *Myc. coeliacum*, to the genus *Flavobacterium*. When the writer, in March, 1931, received cultures of some of the mycobacteria of Gray and Thornton (kindly submitted by Dr. H. G. Thornton and Mr. H. Nicol, Rothamsted Experimental Station) for comparison with a number of similar organisms isolated from Australian soils, an opportunity was found of studying the morphology of *Myc. coeliacum* a little more closely, in order to see whether Bergey's transfer of the organism to a genus of a different family and order (according to Bergey's own system of classification) is justifiable on morphological grounds.

In spite of several years of artificial cultivation the organism was still true to the characters mentioned by Gray and Thornton (1928). On ordinary nutrient agar + 1% dextrose it produces an abundant growth at 22–25° C.,* with smooth, raised, shining surface, undulate margin, first white, later pale pinkish-buff. In gelatin stab the growth is very thin; surface growth yellowish, zonate, with many lobes and secondary colonies along edge; no liquefaction. On synthetic media (dextrose-asparagine-agar and saccharose-nitrate-agar) the growth is similar to that on nutrient agar, but less abundant, with more rugose surface and of drier consistency. Microscopically the organism appears in quite young cultures (20–24 hours) as bent and curved rods, often in V-shaped arrangement, 2–6 × 0·8–1·0 μ. After two days and in older cultures one sees mostly quite short rods and cocci, 0·8–1·2 × 1·0–1·8 μ. The organism is gram positive. Gray and Thornton state

* Bergey's statement: "Optimum temperature 30° to 35° C." is incorrect. Gray and Thornton give the optimum temperature as below 30° C., and in agreement herewith the present strain grew scantily or not at all, according to the medium, at 35° C. The same is true of Bergey's statement: "Aerobic facultative". Gray and Thornton do not say anything concerning this point, but the present strain is obligate and strictly aerobic. No growth takes place in oxygen-free atmosphere, and in shake-culture on dextrose-nutrient-agar no growth is visible below a depth of about 2 mm.

that it is not acid-fast by the Ziehl–Neelsen method. The present strain showed a slight degree of acid-fastness in 20–24 hours old culture on dextrose-nutrient-agar, but not in two days old cultures or on synthetic media. In milk-culture the coccoid forms were fairly acid-fast after three days, although much less than *Myc. tuberculosis* or *M. phlei*.



Text-fig. 1.—Growth of *Myc. coeliacum* on agar, observed directly under the microscope. *a-d*, dextrose-asparagine-agar, 12 hours. *e*, same specimen as *d*, observed two hours later; septa have been formed, and the bottom cell is degenerating. *f*, same medium, 20 hours. *g*, same medium, 48 hours. *h*, water-agar, 20 hours. *i*, the same medium, 48 hours. Magnification: × 1000.

The mode of growth and reproduction of the organism was studied on three different agar media: (1) rich, complex medium: ordinary meat extract-peptone-agar + 1% dextrose, (2) synthetic medium: 1% dextrose, 0·1% asparagine, 0·1% K_2HPO_4, 1·5% agar, and (3) starvation medium: 2% agar in tap water. Cell material from a young culture on medium 2 was smeared out on the surface of sterile agar in petri dishes, and at various intervals of time small blocks of agar were cut out and examined under immersion lenses. The usual convenient method of hanging agar block culture could not be used for continuous observation of the growth, because the development of the cells soon came to a stand-still under the coverslip. The course of development was in all essentials the same on the three media (see Text-fig. 1). After 11–13 hours at 22–24° C., the cells of the inoculum have grown out into fairly long rods and filaments, up to 18–20 μ, showing true branching, occasionally to such an extent as to form small mycelia (Fig. 1, *c*, *d*, *e*). Already at this stage there is a formation of septa, resulting in cell division, and the daughter-cells occupy frequently an angular position (Fig. 1, *a*, *b*, *e*). After 18–20 hours the process of cell-division has gone so far that nearly all evidence of branching has disappeared, and the young colonies appear as composed of irregular, uneven-sided rods, about 1 μ thick and of varying length, mostly 3–8, up to 13 μ long, arranged in a characteristic angular manner and

adjoining each other at the corners, resembling diphtheria bacilli; like these, they also sometimes show parallel arrangement (Fig. 1, *f*). In some cases the young cells are seen "slipping" past each other like tubercle bacilli (Fig. 1, *h*). After two days the long rods have divided further into short rods and cocci, generally showing the same arrangement as the long rods previously (Fig. 1, *g*, *i*); this is best seen on medium 3, where only a very thin growth is produced. The life-cycle of *Myc. coeliacum* does thus, so far studied, and under the present conditions, comprise the following three main forms:

1. Long, branched rods, sometimes approaching a mycelial type.
2. Unbranched, irregular rods of medium length, resembling diphtheroids.
3. Short rods and cocci.

The figure of the growth after 12–24 hours is quite like that described for *Myc. tuberculosis* (Miehe, 1909; Gardner, 1929), saprophytic mycobacteria (Ørskov, 1923; Haag, 1927), and diphtheroids (Graham-Smith, 1910; Ørskov, 1923; Haag, 1927). Both the "snapping" type of growth of the corynebacteria (termed "angular growth" by Ørskov) and the subsequent "slipping" growth of the mycobacteria are seen here, and the occasional formation of what resembles a small mycelium has its parallels in both *Myc. tuberculosis* (Miehe, 1909) and *Myc. phlei* (Ørskov, 1923). Further, the evidence of acid-fastness under certain conditions points towards the genus *Mycobacterium*, although this character is shared to some extent by certain corynebacteria (Haag, 1927). All this speaks definitely against the classification of the organism with the genus *Flavobacterium*; moreover, the yellow pigment which should characterize this genus, is not typical here. Whether it should be termed *Mycobacterium* or *Corynebacterium* may be disputable, and this question cannot be answered satisfactorily until we possess more information concerning the complete life history of these organisms. For the present there seems to be no serious objection to the classification adopted by Gray and Thornton.

Summary.

A study of the morphology of *Myc. coeliacum* showed that this organism agrees morphologically with the genera *Mycobacterium* and *Corynebacterium*. The suggested transfer of it to the genus *Flavobacterium* cannot, therefore, be regarded as justified.

References.

Bergey, D. H., 1930.—Manual of Determinative Bacteriology. Third Ed., Baltimore.

Gardner, A. D., 1929.—Growth and Reproduction of *B. tuberculosis*. *Journ. Path. and Bac.*, 32, 715-716.

Graham-Smith, G. S., 1910.—The Division and Post-Fission Movements of Bacteria when Grown on Solid Media. *Parasitology*, 3, 17-53.

Gray, P. H. H., and Thornton, H. G., 1928.—Soil Bacteria that decompose certain Aromatic Compounds. *Cent. f. Bakt.*, II, 73, 74-96.

Haag, F. E., 1927.—Die saprophytischen Mykobakterien. *Cent. f. Bakt.*, II, 71, 1-45.

Miehe, H., 1909.—Beiträge zur Biologie, Morphologie und Systematik des Tuberkelbacillus. *Zeitschr. f. Hyg.*, 62, 131-154.

Ørskov, J., 1923.—Investigations into the Morphology of the Ray Fungi. Thesis, Copenhagen.